EXHIBIT C

CONFIDENTIAL SUBJECT TO PROTECTIVE ORDER

UNITED STATES INTERNATIONAL TRADE COMMISSION

Washington, D.C.

Before the Honorable Robert L. Barton, Jr.

Administrative Law Judge

In the Matter of

Investigation No. 337-TA-550

Certain Modified Vaccinia Ankara ("MVA") Viruses and Vaccines and Pharmaceutical Compositions Based Thereon

DECLARATION OF PROF. DR. DR. H.C. MULT. ANTON MAYR	ERKLÄRUNG DES PROF. DR. DR. H.C. MULT. ANTON MAYR
I, Professor Anton Mayr, hereby declare and state:	Ich, Professor Anton Mayr, gebe hiermit folgende Erklärung ab:
1. I reside in Starnberg, Germany, and have spent nearly fifty years of my life developing and researching a virus known as Modified Vaccinia Ankara (MVA), which has therapeutic uses, including in vaccines against diseases such as small pox.	1. Ich bin wohnhaft in Starnberg, Deutschland, und habe ungefähr 50 Jahre meines Lebens mit der Entwicklung und Erforschung eines Virus namens Modified Vaccinia Ankara (MVA) verbracht, das therapeutischen Zwecken dient, einschließlich des Gebrauchs von Impfstoffen gegen Krankheiten wie zum Beispiel Pocken.

2. I have a doctorate degree and a professorship in veterinary medicine from the University of Munich, and received various honorary doctoral degrees from the University of Zurich, Technical University of Munich and Veterinary College of Hanover.

2. Ich habe einen Doktor- und einen Professorentitel in Veterinärmedizin inne und habe eine Reihe von Ehrendoktorgraden der Universität Zürich, der Technischen Universität München und der Tierärztlichen Hochschule in Hannover verliehen bekommen.

3. From 1955 to 1959, I was the director of the Department for Infectious and Tropical Medicine and the Bavarian Vaccine Institut, Munich. From 1959 to 1963, I was the president of the Federal Research Institute for Virus Diseases in Animals, Tübingen, Germany. From 1963 to 1991, I was the director of the Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, University of Munich. Since October 1991, I have been a researcher emeritus at the University of Munich.

3. Ich war von 1955 bis 1959 Mitarbeiter des Institutes für Seuchen und Tropenkrankheiten und der Bayrischen Landesimpfanstalt München. Von 1959 bis 1963 war ich Präsident der Bundesforschungsanstalt für Viruskrankheiten der Tiere in Tübingen, Deutschland. Von 1963 bis 1990 war ich Direktor des Institutes für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin der Tierärztlichen Fakultät der Universität München. Seit 1990 bin ich emeritierter Forscher an der Universität München (Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin).

4. I created MVA and all MVA strains

4. Ich habe MVA entwickelt und alle

originate from me. I created MVA, and a strain referred to as MVA-572, created by continuous passages of a Vaccinia Virus in chicken embryo fibroblasts. I am widely recognized and credited as the originator of MVA.

5. Until I gave an exclusive license and ownership rights in MVA, including MVA-572 and its progeny, to a company named Bavarian Nordic, I owned all MVA strains, including MVA-572 and others that are presently on deposit, for example, at the European Collection of Cell Cultures (ECACC). Prior to the transfer to Bavarian Nordic, which occurred over the period from 1996 to 2002, I owned the MVA strains as the product of my research as director and president of the institutes where I have worked.

MVA-Stämme stammen von mir ab. Ich habe MVA geschaffen sowie einen weiteren attenuierten Stamm mit der Bezeichnung MVA-572, geschaffen durch kontinuierliche Passagen eines Vaccinia-Virus Ankara in Fibroblasten von Hühner-Embryos (CEF). Ich bin weithin anerkannt und angesehen als der Schöpfer von MVA.

5. Bis zum Zeitpunkt, in dem ich exklusive Lizenz- und die Eigentumsrechte an MVA, einschließlich MVA-572 und dessen Nachkommenschaft, auf eine Gesellschaft namens Bavarian Nordic im Jahre 2002 übertrug, gehörten mir alle MVA Passagen, einschließlich MVA-572 und andere, die gegenwärtig zum Beispiel bei der European Collection of Cell Cultures (ECACC) hinterlegt sind. Vor der Übertragung an Bavarian Nordic, die über die Jahre 1996 bis 2002 erfolgte, gehörten mir die MVA Passagen als das Produkt meiner Forschung als Direktor und Präsident der Institute, an denen ich gearbeitet habe.

6. I have provided some samples of MVA to individual scientists, upon request, for research purposes, but not for commercial purposes, such as to develop a vaccine product.

6. Ich habe einige Stämme des MVA individuellen Wissenschaftlern auf Anforderung allein für Forschungszwecke zur Verfügung gestellt, nicht jedoch für kommerzielle Zwecke wie die Entwicklung eines Impfstoffes.

7. Upon request, I provided two samples of MVA to Dr. Bernard Moss of the National Institutes of Health (NIH). Specifically, Dr. Bernard Moss of the NIH approached me in 1995 for a sample MVA virus and again in 2001. I provided Dr. Moss with a sample of MVA-575 in 1995 and with MVA-572 in 2001. As is customary among researchers, I provided Dr. Moss with samples for his research purposes only, not for any commercial purpose, such as using it to create vaccine product, or for any third party use. Dr. Moss was not allowed to commercialize MVA. Dr. Moss was not permitted to give out any samples of MVA-572 or its progeny to any third party without express permission from me.

7. Auf Anforderung habe ich zwei Muster des MVA Dr. Bernard Moss des National Institutes of Health (NIH) gegeben. Genauer gesagt hat mich Dr. Bernard Moss vom NIH im Jahre 1995 um ein Muster des MVA Virus gebeten und noch einmal im Jahre 2001. Ich habe Dr. Moss ein Muster des MVA- 575 im Jahre 1995 und des MVA-572 im Jahre 2001 verschafft. Wie es unter Forschern üblich ist, überließ ich Dr. Moss diese Muster nur für Forschungszwecke, nicht für kommerzielle Zwecke, wie die Nutzung zur Schaffung eines Impfstoffes oder für irgendeine Nutzung durch Dritte. Dr. Moss hatte keine Erlaubnis zur kommerziellen Nutzung des MVA. Dr. Moss war nicht gestattet, Muster von MVA-572 oder seiner Nachkommenschaft an einen Dritten

ohne meine ausdrückliche Zustimmung herauszugeben.

8. A company called Therion approached Dr. Moss to receive a sample of MVA-572 around February of 2002. Therion Biologics of Cambridge, Massachusetts wrote a letter to me indicating that Dr. Moss would not provide MVA-572 to Therion without permission from me.

8. Eine Gesellschaft namens Therion wandte sich um den Februar 2002 an Dr. Moss, um ein Muster des MVA-572 zu erhalten. Therion Biologics of Cambridge, Massachusetts schrieb einen Brief an mich mit dem Inhalt, dass Dr. Moss kein MVA-572 an Therion ohne Erlaubnis durch mich weitergeben würde.

9. At some point after September 11, 2001, Dr. Moss decided no longer to honor his agreement with respect to MVA samples provided by me and decided to take my MVA sample and use it as the basis for a commercial, small pox vaccine product. I sent letters to Dr. Moss advising against such use of the MVA sample. I believe that Bavarian Nordic, the present owner, also sent letters and attempted to stop Dr. Moss and the NIH from unauthorized commercial use of the MVA-572 or its progeny. MVA-572 and its progeny are owned by Bavarian Nordic and no commercial

9. Zu einem Zeitpunkt nach dem 11. September 2001 entschied sich Dr. Moss, die Vereinbarung hinsichtlich der MVA Muster, die er von mir bekommen hatte, nicht mehr einzuhalten, nahm meine MVA Muster und nutzte sie als Grundlage für einen kommerziellen Pocken-Impfstoff. Ich schrieb Dr. Moss Briefe, in denen ich ihm von einem solchen Gebrauch der MVA Muster abriet. Ich glaube, dass Bavarian Nordic, die gegenwärtige Eigentümerin, ebenfalls Briefe geschrieben hat und versucht hat, Dr. Moss und das NIH von der unbefugten

use of this strain is permitted by any other party without their permission.

kommerziellen Nutzung des MVA-572 und seiner Nachkommenschaft abzuhalten. MVA-572 und seine Nachkommenschaft gehören Bavarian Nordic und ein kommerzieller Gebrauch dieses Stamms durch eine andere Partei ohne Erlaubnis von Bavarian Nordic ist nicht gestattet.

I declare upon penalty of perjury under the laws of the United States that the foregoing is true and correct.

Executed this ____ day of November, 2005.

Ich erkläre unter Eides Statt nach den Gesetzen der Vereinigten Staaten, dass die vorstehende Erklärung der Wahrheit entspricht.

Ausgefertigt am 14. November 2005.

Respectfully Submitted,

B. Ruiz

Prof. Dr. Dr. h.c. mult. Anton Mayr

EXHIBIT D

REDACTED IN ITS ENTIRETY

EXHIBIT E

A. Mayr, V. Hochstein-Mintzel, H. Stickl

Passage History, Properties, and Use of the Attenuated Vaccinia Virus Strain MVA

Zusammenfassung: Abstammung, Eigenschaften und Verwendung des attenuierten Vaccinia-Stammes MVA. Das MVA-Virus repräsentiert ein Laborvirus, das sich durch zahlreiche biologische Marker von den bekannten Vaccinia-Stämmen wie auch von den anderen Viren der Orthopox-Gruppe sicher differenzieren läßt. Es kommt nicht in der Natur vor und besitzt für Mensch und Tiere bei fehlender Kontagiosität nur noch eine geringgradige Virulenz. Es kann ohne Gefahr sowohl parenteral wie auch lokal, insbesondere oral und intrakutan, appliziert werden. Nach lokaler Verabreichung induziert es sehr stark die Bildung von endogenem Interferon. Voraussetzung hierfür ist die Impfung mit hohen Virusdosen (über $10^{7,5}$ FHE-KID^{50}/ml).
Mit dem MVA-Virus steht ein Impfvirus zur Prophylaxe und Bekämpfung von Orthopox-Viruserkrankungen zur Verfügung, das in der Human- und Tiermedizin gleichermaßen ohne Schaden für den Impfling wie für die Umgebung verwendet werden kann. Es eignet sich auch für Inkubations- und Notimpfungen.

Summary: Vaccinia virus strain MVA is derived from Vaccinia virus strain Ankara through 530 continuous passages in cell cultures of chick embryo fibroblasts. Strain MVA can be differentiated from all known strains of vaccinia virus and other members of the orthopox group. It does not occur naturally, and is of low virulence for man and animals; local and parenteral applications are innocuous. This particularly applies to oral and intracutaneous administration. Strain MVA strongly induces local endogenous interferon if applied to the mucous membranes in concentrations above $10^{7.5}$ CEF-ID_{50}.
It also induces clinical immunity against diseases caused by orthopox viruses, including experimental smallpox. Repeated revaccinations are innocuous and strongly enhance the immunizing effect.
Strain MVA primarily stimulates cellular immunity; antibody production is less prominent. Cellular defense is of primary importance in pox virus immunity.

Introduction

Together with the variola and alastrim viruses and 8 more animal pox species (cowpox, buffalopox, rabbitpox, horsepox, elephantpox, mousepox, monkeypox, and camelpox viruses), the vaccinia virus forms the very uniform viral group of orthopoxviruses which belongs to the family of "Poxviridae". In their morphological and physicochemical properties, the Orthopoxviridae are largely identical. According to the established categorization of viruses, they belong to the same serotype (21). Each of the 11 viral types can be used for cross immunization against the heterologous pox disease, although the original human and animal pox viruses will provide for better immunization. The vaccinia virus is an exception providing equally good immunization against all of these diseases.
In addition, the vaccinia virus has the broadest spectrum of activity. It is infectious for humans as well as for animals. Among mammalian species, the infection spreads particularly easy among cows, pigs, horses, donkeys, sheep, goats, rabbits, monkeys, buffaloes, camels, elephants, and zoo animals. Natural infections are possible but extremely rare. Usually animals will acquire the diseases in connection with smallpox vaccinations of humans. The infections may cause local or generalized disease, with the local form predominating. Generalized vaccinia pox disease will preferably occur in horses, camels, elephants, pigs, rabbits, and young animals.
The other orthopoxviruses, on the other hand, are fairly specific for their host. Variola and alastrim viruses will cause human pox, the animal pox viruses causing the respective

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Prof. Dr. Dr. *A. Mayr*, Institut für Mikrobiologie und Infektionskrankheiten der Tiere der Universität, D-8000 München 22, Veterinärstr. 13; Dr. *V. Hochstein-Mintzel*, Prof. Dr. *H. Stickl*, Bayerische Landesimpfanstalt, D-8000 München, Am Neudeck 1.

original diseases in cows, monkeys, camels etc. While under natural conditions the variola or alastrim viruses will not be transferred from humans to animals (9), with the exception of the ectromelia virus the original animal pox viruses can be transmitted to humans by intensive direct or indirect contact. In general they will then cause a benign local disease. Moreover, in highly susceptible children and in individuals particularly disposed, they may cause severe generalized disease. Irrespective of their course, they are, however, characterized by their contagiousness being low or absent.

To date, prophylactic immunizations against smallpox disease in humans and animals are only possible using live vaccines. The mechanism responsible for specific protection appears to be based on the formation of immune cells as well as on humoral defense parameters. Being able to always use the same vaccine virus for active immunization against all diseases caused by orthopoxviruses without any risk to humans, animals, or the environment would be ideal. Currently, the vaccinia strains used for smallpox vaccinations in humans do not meet these requirements. This also applies to the strain *Elstree* which is recommended by the WHO (*cf.* above).

We, therefore, tried to attenuate the vaccinia virus by continuous passages in various cell systems while preserving its immunologic characteristics so that it meets the above-mentioned requirements. At the same time we tried to achieve a vaccine virus which will allow for innocuous parenteral or oral vaccination inducing rapid production of endogenous interferon, not being contagious for humans or animals, not being found in animals in a natural environment, and, finally, allowing for non-hazardous incubation vaccinations when applied locally.

The attenuation of field viruses with the object to grow vaccine strains for the production of vaccines is not a new procedure. Numerous established live vaccines being used all over the world in human and veterinary medicine are a result of this procedure. There is fairly little experience on the attenuation of vaccinia virus and its use as a vaccine in humans, although *Rivers and collaborators* drew attention to this as early as in 1931 (23, 24, 26). They passed the vaccinia virus on the chorioallantoic membrane of chick embryos. In 1968, *Kempe* continued these investigations and successfully used such an "egg virus" for the vaccination of eczema patients (12). Attenuating the vaccinia virus in cell cultures has also been attempted (1, 3, 4, 13, 22, 23). In each case, changes in certain biological properties of the vaccinia virus associated with a decrease its virulence for laboratory animals resulted. Recently, a survey of these works was presented (10).

1. Passage history of the MVA virus and attenuation

The MVA virus was derived from the dermovaccinia strain CVA. It was maintained for many years in Turkey (Ankara Vaccination Institute) on donkey–calf–donkey passages and served there as the basis for the human smallpox vaccine. The raw material was the harvest from pustules of the donkey passages. In 1953, we purified the strain (fractionated ultracentrifugation) and submitted it to two passages on cattle (cutaneous area vaccination). It was subsequently made publicly available as smallpox vaccine in the Federal Republic of Germany in the years 1954/55 (8). During this time, we examined the CVA virus together with other domestic and foreign dermovaccines, neurovaccines, and testicular vaccines, comparing them to other animal viruses with respect to their serologic and biologic differences.

Initially, we investigated the behavior of the viral strains in 10-days-old chick embryos after inoculating the chorioallantoic membrane (CAM) and in rabbits after intradermal (I.D.) and intravenous (I.V.) vaccination. In the egg the CVA virus was very virulent and characterized by:

1. flat primary and secondary lesions with clearly defined contours and broad, deep central necrosis,
2. 100% 4+ generalization
3. a tendency for secondary pock formation
4. a strong vascular effect without hemorrhagic element

5. 100% mortality rate with fairly early generalization
6. skin pocks in the chick embryo.

In the rabbit, the CVA strain behaved inversely. It was fairly histocompatible. Following intradermal vaccination, well-defined infiltrations developed without central necrosis, without hemorrhagia, without secondary pocks in the absence of generalization and with rapid remission. Intravenous application caused short-term fever (2 to 3 days) without producing visible generalization in skin or mucous membranes (6, 7).

In mice aged 3 to 5 days, the CVA virus resulted in generalized pox disease following intraperitoneal application, resulting in the death of the animals. The virus had a very broad spectrum of activity on the cellular level. It proliferated in high titers and caused lysis in all cell cultures (8, 22, 28).

Various vaccinia strains (egg virus, culture virus) agglutinated identical chick blood cells to various degrees irrespective of their infectious titers. Strain CVA had good hemagglutinating activity (16).

When applied in humans (primary vaccinees), strain CVA did not differ from the other dermovaccinia strains with respect to local and general vaccine reactions. Percentages of the various postvaccinal complications were also similar. However, there was a significant difference with regard to the propensity of the CVA virus to cause the formation of secondary pocks and the late shedding of crusts of vaccinia lesions. In the catchment area of the Munich Vaccination Institute during the years 1954/55, reports of secondary pocks were received for 13 cases for strain CVM, 50 cases for strain CVB, and in contrast 781 cases for strain CVA, with approximately the same numbers of primary vaccinees (8). As a result, the Munich Vaccination Institute no longer used strain CVA in the production of smallpox vaccines during the following years.

We considered strain CVA particularly suitable for our attenuation experiments as it has typical biologic "markers": histocompatible behavior in the rabbit, rapid and intense generalization in the incubated chicken egg, intense and broad development of central necrosis in primary and secondary lesions on the CAM with fairly mild mesenchymal reaction, skin pocks in the chick embryo, severe virulence in infant mice, broad spectrum of activity in cell cultures with lysis of the affected cells, good hemagglutinating and immunizing activity, and, finally, tendency to cause secondary pocks and late shedding of crusts of the vaccination pustules in humans.

In 1958, we initiated our attempts at attenuation by submitting the CVA virus (cell-free virus) to continuous passages by the serial dilution technique in various primary cell cultures (tubes containing the last dilution in the series). After 300 passages, we determined by comparisons that the virus in the chick embryo fibroblast (FHE) cell passages showed greater changes than in the cell cultures of mammalian cells (calf and porcine kidney cultures). Therefore, we continued submitting the CVA virus to passages on FHE cultures. To prepare the cultures, we used eggs from a health-controlled chicken farm. Sterile bovine amniotic fluid was used as a medium (method by *Mayr* and *Kolcher*, 1960 [17]). The virus was always harvested at the peak of the cytopathic effect. Non-inoculated controls were checked for unintentional viral contamination. If there was any doubt, the respective cultures were discarded. After 360 passages the virus was cloned by the plaque method. For this purpose, three consecutive plaque passages were performed with the material of the isolated plaque always being titrated in dilutions on the new plaque dish and for each further passage dishes with no more than one plaque each being used (serial dilution technique) (20).

In 1963, we examined passage 370/371 of the FHE virus with regard to morphologic, serologic, and biologic parameters versus the CVA basic virus and other dermovaccinia and egg vaccinia strains (22). The typical vaccinia nature had disappeared completely. Only with regard to morphology, the FHE passage virus had any similarity with the original virus. The most remarkable characteristic was the loss or the severe decrease in virulence for the rabbit, the infant mouse, and for certain cell cultures. The chick embryo (CAM inoculation), too, no

longer showed any typical vaccinia properties. Rather than flat lesions with a deep central necrosis (original virus), the FHE virus produced small compact proliferation nodules without necrosis. The virulence (vascular damage, involvement of the surroundings, mortality rates) was also significantly decreased as compared to the controls while the propensity for generalization was retained. In the cross neutralizing test it was neutralized by specific vaccinia immune sera. Its hemagglutinating activity was decreased. Controls for foreign virus contamination were negative.

Since that time, the CVA-FHE virus was submitted to further passages on FHE cultures. By now, culture passage 570 has been reached and the virus appears to be genetically uniform and stable. The last passages were again cloned by the serial plaque dilution technique. The eggs used for the plaque dishes originated in an approved leukosis-free fowl population. After clinical testing in humans, the CVA-FHE virus was designated **MVA virus**—**m**odified **v**accinia virus **A**nkara—starting with the 516th FHE passage, due to its stability and its changed properties and in order to avoid confusing it with other attenuated vaccinia strains (10, 27).

2. Properties of the MVA virus

In its morphology and structure, the MVA virus resembles the general composition of viruses of the orthopox group. With regard to serology and immunobiology, it also belongs to the serotype typical of orthopoxviruses. However, the MVA virus has stable biological markers, allowing for differentiation from other species of orthopoxviruses.

The MVA virus can be clearly differentiated from the other vaccinia strains and the other species of orthopoxviruses by its pathogenic behavior:

1. in the chick embryo after CAM inoculation = *CHE marker*
2. in various cell cultures = *TC marker*
3. in the rabbit = *R marker*
4. in the infant and adult mouse = *M marker*
5. in the hen = *F marker*
6. in the monkey = *MK marker*
7. in humans = *H marker*

In addition to the above-mentioned markers, the MVA virus has the ability to induce the production of endogenic interferon to a particularly high degree. In addition to the production of interferon, the process is associated with a significant increase in the rate of phagocytosis. Interferon induction is more pronounced when large quantities of the virus (more than $10^{7.5}$ FHE $TCID_{50}$/ml) are administered than when low doses are used for vaccination.

The production of endogenic interferon was tested in the rabbit. The rabbits received three doses of 0.2 ml of MVA virus each intranasally at intervals of several hours (titer $10^{7.5}$ FHE $TCID_{50}$/ml). Blood samples were taken from the animals prior to the application and 6, 12, 24, and 48, 72, 96 hours after the application. The interferon content of the serum (acidified over night to pH 2) was determined. The analysis was performed using the plaque inhibition test in RK-13 cell cultures according to the established methods. Sindbis virus, strain AR 86, grown in the respective cell cultures, was used as test virus. The diagnosis of viral use was 50-80 plaque-forming units. The interferon activity of the samples was measured in interferon units per ml (effective dose 50% = ED_{50}). An ED_{50} is the reciprocal of the last stage of dilution of the material under investigation in which a reduction in the number of plaques of at least 50% from the respective controls is observed. Prior to treatment, the serum of the animals was negative. As early as after 6 hours we found average serum interferon measurements of 16 to 64. Interferon titers (ED_{50}/ml) then increased continuously until day 3 to reach measurements of 256. On day 4, the titers then decreased slightly.

In addition to the induction of endogenous interferon, we examined the production of interferon in chick embryo fibroblast cell cultures after incubation with UV-inactivated, high-passage virus. Using vaccinia virus from the late cell culture passages (passages 428-458) better interferons could be achieved after UV-inactivation of equal quantities of virus

than with virus strains taken from the earlier culture passages (passages 11 and 12). Interferons from the UV-inactivated virus from the later passage achieved measurements of 1024 ED_{50}/ml against the Sindbis virus (plaque test with 20-50 plaque-forming units) while measurements for UV-inactivated virus from the earlier passage were 64-128 (17).

Increasing the rate of phagocytosis was tested in the mouse phagocytosis test (NMRI mouse). The method is based on measuring the rate of elimination of coal particles from the circulating blood. The animals were vaccinated with 0.3 ml of MVA virus I.P. each (titer $10^{7.5}$ FH_E-$TCID_{50}$/ml). After 48 hours, we performed the test according to *Buschmann* and collaborators (2). In the control group (30 animals), the phagocytosis index K indicating the rate of elimination of the coal particles from the blood was fairly constant with measurements between 0.029 548 and 0.031 089. In the experimental animals (3 groups of 30 animals each) it was significantly increased at 0.040 811 to 0.043 212.

Tables 1, 2, and 3 provide a survey of the various biological markers of the MVA virus.

The MVA virus is an artificial laboratory product which is not identical with any of the known, naturally occurring orthopoxvirus species and does not occur naturally. It can be well differentiated from all these types of viruses. The F marker indicates that it originated in the vaccinia virus and shows its vaccinia characteristic. When applied cutaneously, the MVA virus will cause a mild follicular reaction (defined swelling of the follicles without

Table 1: *CHE and TC markers of the MVA virus as compared to the CVA dermovaccinia basic virus*

Marker		MVA virus	CVA basic virus
Type	Reference		
CHE inoculation of CAM of 10-day-old chick embryos, 37°C incubation, reference: day 4 post infection	*p:* characteristics of primary plaques	A: small compact proliferation nodules without central necrosis	A: flat plaques with deep, wide central necrosis
	m: percentage of necrosis rate	B: 40	B: 100
	g: percentage of generalization	B: 100	B: 100
	qu: quantity of generalization	B: + until + + +	B: + + + +
	op: other properties	Ø	Secondary pocks, skin pocks in the embryo
TC inoculation of primary cell cultures and cell lines, reference: 10^3 FHE-$TCID_{50}$/0.1ml	*fhe:* chick embryo fibroblasts	V: + + + + L: + + + + CPE: small rounded cells with granular decay	V: + + + + L: + + + + CPE: plaque-shaped degeneration, cell fusion, lysis
	pk: porcine kidney cells	V: + + L: (plaque)	V: + + + + L: + + + +
	kk: calf kidney cells	V: ± L: O	V: + + + + L: + + + +
	kt: calf testicular cells	V: ± L: O	V: + + + + L: + + + +
	h: HELA cells	V: ± L: O	V: + + + + L: + + + +

Note: A = 10-15 FHE-$TCID_{50}$/0.1 ml; B = $10^{3.0}$ FHE-$TCID_{50}$/0.1 ml; V = viral proliferation; L = cell culture lysis; CPE = type of the cytopathic effect

Table 2: *R, M, F markers of the MVA virus as compared to the CVA dermovaccinia basic virus (reference value $10^{6.0}$ FHE $TCID_{50}$/ml)*

Marker			MVA virus	CVA basic virus
Type	Reference			
R Rabbits, German White Giant rabbits, 6 months old	*iv*	intravenous, 1.0 ml	G Ø	G Ø
	ik	intradermal, 0.1 ml	P Ø	P + + + +
	k	cutaneous, scarification method, 0.05 ml	P Ø	P + + + +
M infant (1-3 days old) and adult mouse (12-15 g)	*ip*	infant mouse, intraperitoneal, 0.1 ml	M Ø	M 80
	ic	infant mouse, intracerebral, 0.05 ml	M Ø	M 100
	ica	adult mouse, intracerebral, 0.1 ml	M Ø	M 100
F chicks and young hens	*k*	cutaneous follicular inoculation, 0.05 ml	*chick:* P Ø *hen:* P +	*chick:* P + + + *hen:* P + + +

Notes: G = percentage of generalization
P = intensity of primary reaction at the vaccination site
M = percentage of fatal outcome

area) in young hens (follicular method), which according to *Mayr* is typical for vaccinia viruses (18). In non-attenuated vaccinia strains the local follicular reaction will take a significantly more severe course and will also occur in chicks.

The most susceptible host system for the MVA virus is the chick embryo after inoculation of the CAM. In the chick embryo (CHE marker) the MVA virus is characterized by small, compact proliferation nodules often drawn out to the shape of a semicolon (primary and secondary lesions) with a small opacified margin and without central necrosis. The virulence for

Table 3: *MK and H markers of the MVA virus as compared to the CVA dermovaccinia basic virus (reference value $10^{6.0}$ FHE $TCID_{50}$/ml)*

Marker			MVA virus	CVA basic virus
Type	Reference			
MK monkeys, Macaca irus, age not defined	*ik*	0.2 ml	P Ø	P + + + +
	k	0.05 ml	P Ø	P + + + +
	b	buccal, 0.1 ml	P Ø	P + + + +
	ith	intrathalamic, 0.1 ml	MB Ø	MB 100
H man, primary vaccinee	*ik*	0.2 ml	P + redness, mild infiltration up to 20 mm	P + + + + severe reaction with formation of pustules and necroses
	i.m.	intramuscular, 0.2 ml	P Ø	not examined
	k	epicutaneous cut	P Ø	P + + + + severe normal primary vaccination reaction

Notes: *cf.* Table 2
MB = percentage of diseases

the chick embryo was not lost in the course of the continuous passages on FHE cultures, although it was clearly reduced. Up to day 4 post infection, the time when the original CVA virus will 100% generalize with a mortality rate of 100%, only 40% of the embryos had died after infection with the MVA virus the generalization rate being 100%. The time point of generalization was delayed by 20 to 24 hours. The quantity of generalization depends on the quantity of vaccine virus used. At vaccine doses under 10^2 $TICD_{50}$ it is +, however, at an inoculation quantity of more than 1000 it is +++. It lost its ability to cause secondary pocks and skin pocks in the embryo. Figures 1 and 2 show the different pictures of the chorioallantoic membrane of the primary and secondary lesions for the original CVA virus (Fig. 1) and the MVA virus (Fig. 2).

The most prominent characteristic of the MVA virus on the cellular level (CTC marker) is the dramatic narrowing of the host spectrum. The MVA virus is only fully virulent for chick embryo fibroblast cultures, where it differs from the original CVA virus in the quality of the cytopathic effect. Similar to the other vaccinia virus strains, the original CVA basic virus will cause plaque-shaped degeneration (flat spreading of the infected cells) with cell fusions and eventually lysis of the infected cell cultures. There is no typical rounding phase (Fig. 3). In contrast, in the MVA virus, there will be rounding (very small and irregular globules) of the infected cells which is followed by granular decay with the granular cell detritus being preserved (Fig. 4). This is particularly noticeable in the plaque test (cf. table 1). In very dense cultures, the cell lawn may be preserved

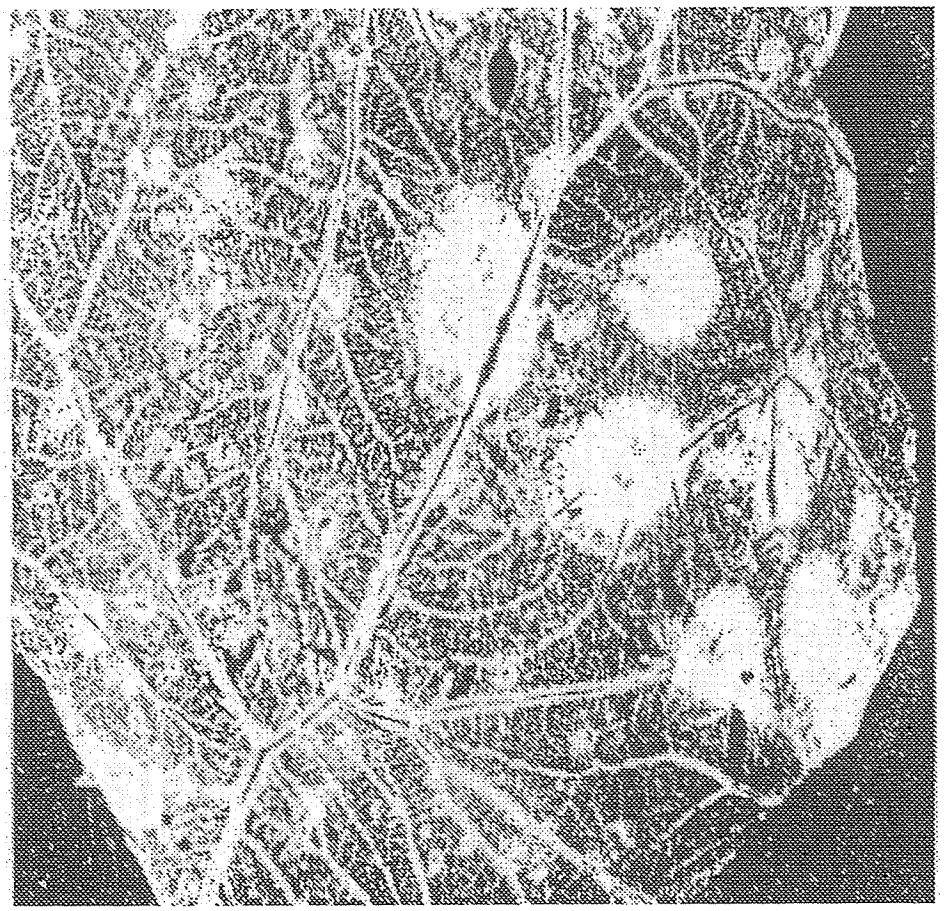

Figure 1: *Chorioallantoic membrane—image of the primary and secondary lesions for the CVA basic virus: day 4.*

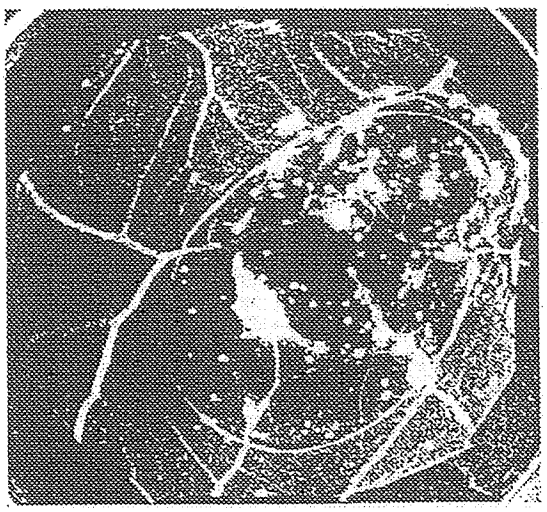

Figure 2: *Chorioallantoic membrane—image of the primary and secondary lesions for the MVA virus: day 4 post infection.*

in its pathologically changed state (rounding phase).

In the rabbit (R marker) and in the mouse (M marker), too, the MVA virus lost its vaccinia characteristics completely. In the rabbit, cutaneous and intradermal vaccinations did not cause a primary reaction at the injection site, and with intravenous application no generalization will develop.

In the mouse, the most noticeable characteristic is the loss of neurovirulence. Both infantile (1-3 days old) and adult mice (12-15 g) did not develop the disease after intracerebral application. Following intraperitoneal vaccination of infant mice aged 1-3 days there generalization is absent (*cf.* Table 2).

Macaca monkeys infected intradermally and buccally (MK marker) did not show any primary reactions. Monkeys infected intrathalamically will not develop disease, while of those monkeys infected with the CVA basic virus and other vaccinia viruses, or even with the strain Elstree, 100% will develop disease (*cf.* Table 3). Finally, the significant loss in virulence of the strain MVA is particularly noticeable in humans (H marker). With regard to primary reactions at the injection site, the course of the usual vaccination by epicutaneous cut or prick is negative. Nor will intramuscular application of MVA virus cause any local reactions. Only after intradermal application, a mild infiltration of up to 20 mm which is clearly demarcated from its surroundings and redness in the area will develop. The reaction will rapidly disappear without any loss of substance or formation of scabs (*cf.* Table 3). There are no general reactions.

With regard to antigen structure and immunogenic properties the MVA virus has not changed. In the purified state (achieved by fractionated ultracentrifugation) it is neutralized by specific vaccinia immune sera irrespective of their origin. Due to its significant loss in virulence for mammals, it will only have immunogenic activity in the vaccinee if multiple doses with high concentrations (more than $10^{7.5}$ FHE $TCID_{50}$) are applied. In this respect, the MVA virus behaves similar to an inactivated virus although there is a difference in that it will stimulate the formation of immune cells which are essential for immunity against smallpox in addition to stimulating the formation of antibodies. A precondition for the vaccination success is a good absorption of the virus. Parenteral, oral, intranasal, or intradermal application of the MVA virus will, therefore, support the success of the immunization better than cutaneous application.

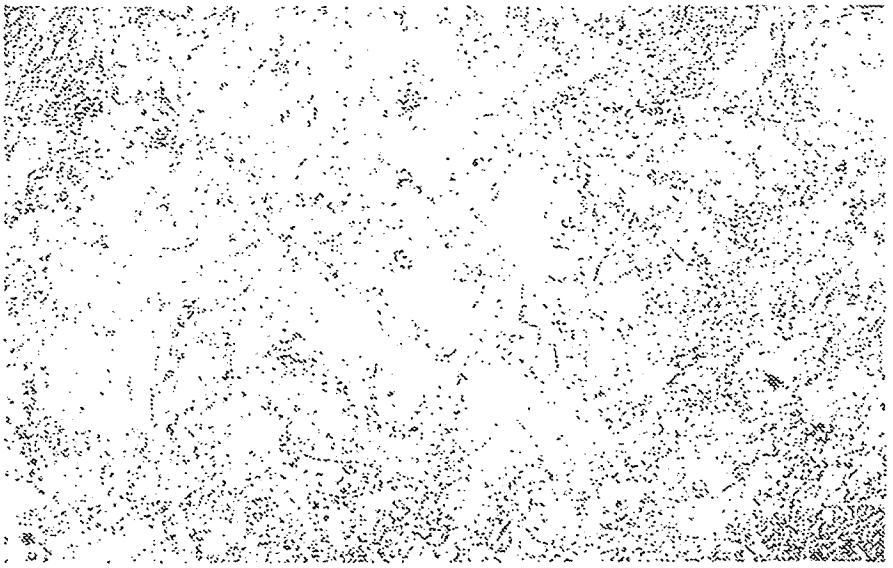

Figure 3: *Cytopathic effect of the CVA basic virus in chick embryo fibroblast cultures: day 2 post infection.*

3. Use of the MVA virus

The MVA virus is suitable for active immunoprophylaxis against all human and animal diseases caused by orthopoxviruses. Due to its low virulence and its intense and rapid induction of the production of endogenous interferon, the MVA virus can be used safely in emergency vaccinations as well. The MVA virus is not contagious. Therefore, it is impossible for vaccinated humans or animals to transmit the virus to susceptible individuals, irrespective of the route of application employed in the vaccination.

The safety of the MVA virus with regard to domestic animals which are significantly more susceptible than humans was demonstrated in newborn, germ-free animals bred by gnotobiotic methods and in conventional newborn animals.

For the experiments with gnotobiotes we used piglets born by cesarean section under sterile precautionary conditions. They were immediately transferred to an isolation system where they received sterile feed. Starting from the time of birth, they received daily oral (in the milk) doses of $10^{7.2}$ FHE $TCID_{50}$ of MVA virus over a period of 10 days. In parallel to these, untreated controls were kept under identical conditions. Health as well as comparative weight gain monitoring was performed and the animals were gradually conventionalized after 14 days. A total of 26 piglets were challenged with the MVA virus this way. None of the animals developed disease or showed any abnormalities. Weight gain was slightly better in the animals vaccinated with MVA as compared to the controls. Vaccinated animals and controls tolerated conventionalization equally well. In the vaccinees, development and condition were even better than in the untreated piglets.

In addition to this experiment, we challenged conventional newborn calves, piglets, and dogs with the doses of MVA virus indicated above. The calves and piglets received the virus over the course of several days (2 to 10 times daily) via the oral route (in the feed or by gastric tube), the pups received one oral or intraperitoneal vaccination. For the vaccination period, all animals remained in the mother population which also contained unvaccinated newborn animals as controls. A total of 18 newborn piglets, 100 newborn calves, and 10 newborn pups were vaccinated. None of the animals developed disease, control animals were not at risk. In addition, two piglets aged 14 days received 5 ml of MVA intravenously ($10^{5.0}$ $TCID_{50}$/ml). The animals tolerated the dose without any reactions.

In veterinary medicine, we so far used the MVA vaccine for quite different purposes. Repeatedly, mouse breeds are endangered by ectromelia endemics. For one year, we have been vaccinating several of these laboratory animal breeds (more than 1000 founder animals per breed) with MVA as prophylactic treatment against mousepox. All dams and sires older than 3 weeks are vaccinated. The vaccination is performed by the intraperitoneal route using 0.2 ml of vaccine. The vaccine contains $10^{7.5}$ FHE $TCID_{50}$/ml. Vaccinations are performed once a year. There were no cases of infection with the vaccine pathogen or breakdown of vaccine immunity due to the introduction of ectromelia field virus. The animals developed normally.

Elephants in captivity are particularly susceptible to vaccinia and cowpox infections. They will develop generalized disease with a fairly high rate of lethality. A variola outbreak among circus elephants (5) in the Stuttgart area, during which two animals developed generalized disease and of which the elder elephant died, caused us to vaccinate the remaining eight animals, mostly young animals, with the MVA virus after we had identified the pathogen as vaccinia virus (two contacts in humans). This was undoubtedly an incubation or emergency vaccination. Each animal received 2 ml of vaccine ($10^{7.0}$ FHE $TCID_{50}$/ml) applied subcutaneously at the base of the ear. The animals tolerated the vaccination well. Neither local nor general reactions were observed. None of the animals subsequently acquired variola disease. Meanwhile, as a result of this experience, six young elephants in the Gelsenkirchen zoo and eight elephants in the Berlin zoo were vaccinated in the way described above. The animals in the Berlin zoo received 5 ml rather than 2 ml subcutaneously. All animals tolerated the vaccination without any complications. In the Berlin zoo, one animal developed a fist-sized swelling at the injection site. This is assumed to be a local abscess caused by contamination during the vaccination process.

In addition to the above-mentioned indications, we also vaccinated eight horses, ten head of cattle, and six sheep with MVA virus (2.0 ml, $10^{7.8}$ FHE $TCID_{50}$/ml) by the subcutaneous route. None of the animals showed any local or general reactions. Finally, we used the MVA virus as a biological agent for the induction of interferon in so-called problem populations of calves. In some cattle breeding premises hospitalism will cause high mortality rates among newborn calves during their first few weeks of life. In most cases mixed infections are responsible involving in particular E. coli,

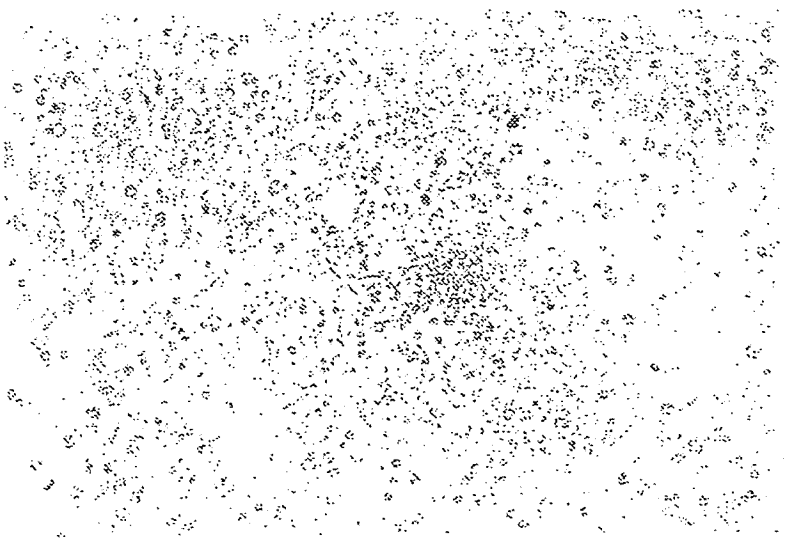

Figure 4: *Cytopathic effect of the MVA virus in chick embryo fibroblast cultures: day 2 post infection.*

pasteurellae, staphylococci, and entero, rhino, adeno, and REO viruses. At the age of 1-4 days, the calves received 1-2 oral doses of 5 ml of MVA virus ($10^{7.5}$ FHE $TCID_{50}$/ml) in the milk at an interval of 24 hours. In none of the animals any postvaccinal reactions were observed. Mortality and morbidity could be significantly reduced.

On the application of the MVA virus in humans, we provided a separate report (29).

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